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A CONVENIENT METHOD TO SYNTHESIZE N-[^{3}H]METHYL-N-NITROSOCARBAMATE TRANSFER REAGENTS

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SUMMARY

Activated N-alkyl-N-nitrosocarbamates are useful acyl transfer reagents that are employed in the synthesis of N-alkyl-N-nitrosoureas and related N-nitroso compounds. The nitrosourea products are of chemical and biological interest because they provide access to the in situ generation of highly reactive carbonium type intermediates, which, depending on their structure, can be powerful carcinogens or antineoplastic agents. The availability of radiolabeled nitrosoureas greatly facilitates studies on their chemical and biological activities. Generally, the synthesis of activated nitrosocarbamates requires condensation of radiolabeled alkylisocyanates with the appropriate alcohol. Because radiolabeled alkylisocyanates are not commercially available and/or troublesome to synthesize, we have developed an easy and economical method for preparing N-[^{3}H]methyl-N-nitrosocarbamates suitable for use as transfer reagents utilizing 1,2,2,2-tetrachloroethyl chloroformate and [^{3}H]methylamine hydrochloride as starting materials.

Key words: N-[^{3}H]alkyl-N-nitrosoureas, N-[^{3}H]alkyl-N-nitrosocarbamates, [^{3}H]methylamine, carcinogenicity

INTRODUCTION

Depending on the alkyl substituent, N-alkyl-N-nitrosoureas are potent genotoxic agents that can be either useful clinical antineoplastic agents, e.g. N-,N'-bis(2-chloroethyl)-N-nitrosourea (1) or potent chemical carcinogens, e.g. N-methyl-N-nitrosourea (2). Under most conditions the extent of DNA modification is quite low and the elucidation of specific DNA adducts is greatly facilitated by the use of radiolabeled material. With a few exceptions, neither the radiolabeled nitrosoureas nor

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convenient starting materials are commercially available. A facile route to many complex nitrosoureas is via the condensation of a radiolabeled N-alkyl-N-nitrosocarbamate with the appropriately substituted amine (see **Scheme 1**) (3-5). Due to the intrinsic regioselectivity of this approach there is no need to separate the two regioisomers that are generated by the direct nitrosation of urea derivatives.

The preparation of radiolabeled N-nitrosoacyl transfer reagents requires the availability of suitably labeled starting materials. In a non-radioactive synthesis this would most often be an alkylisocyanate which can readily be condensed with the appropriate alcohol, e.g. N-hydroxysuccinimide, pentafluorophenol (3). However, radiolabeled alkylisocyanates are not directly available and must be prepared using reactions that are not convenient for radiolabeled synthesis and/or result in significant loss of material (3). We describe herein the synthesis of a convenient transfer reagent, 1,2,2,2-tetrachloroethyl N-[^{3}H]methylcarbamate prepared from [^{3}H]methylamine hydrochloride and 1,2,2,2,-tetrachloroethyl chloroformate (6) and demonstrate that this chloroformate is an excellent choice for the preparation of radiolabeled acyl transfer reagents. The nitrosation of the radiolabeled acyl transfer reagent with nitrogen tetroxide and the subsequent preparation of a N-nitrosourea is also described.

EXPERIMENTAL

Materials

[^{3}H]Methylamine hydrochloride was purchased from Amersham (specific activity, 48Ci/mmol). All purchased chemicals used were of reagent grade, and when necessary, solvents were dried. The 1,2,2,2-tetrachloroethyl chloroformate was purchased from Aldrich, and the methidium amine hydrochloride (8) was prepared using the procedure previously described (4). NMR spectra were run on a Varian XL300 and ^{3}H was measured on a Beckman model LS 3801 scintillation counter.

Succinimidyl N-methylcarbamate (3a)

To a stirred solution of N-hydroxysuccinimide (1a) (1.19 g, 13.07 mmol) in anhydrous dioxane at 0 °C under an argon atmosphere was added bis(trichloromethyl)carbonate (2.3 g, 7.7 mmol) and diisopropylethylamine (1.98 g, 15.03 mmol). After the addition, the reaction mixture was stirred

at room temperature for 2 h and then cooled to -20 °C. A solution of methylamine hydrochloride in dioxane was slowly added and stirring was continued at -20 °C for 2 h and then at room temperature for 24 h. The solvent was evaporated and the resulting residue crystallized from EtOAc to give pure product. Yield, 861 mg (65%), mp 148-149 °C, [Lit. 148-152 °C (3)].

Pentafluorophenyl N-Methylcarbamate (3b)

Similarly, pentafluorophenyl N-methylcarbamate was obtained using the same method as detailed above except pentafluorophenol (1b) was used rather than the succinimide. Yield, 872 mg (35%); mp 123-124 °C, NMR ($CDCl_3$) 2.85(d, 3H, N-CH_3), 5.1(bs, 1H, NH); MS (FAB) m/z 241 (M) and 242 (M+1).

1,2,2,2-Tetrachloroethyl N-methylcarbamate (5)

Methylamine hydrochloride (670 mg, 10 mmol) in dry dioxane (25 ml) and 1,2,2,2-tetrachloroethyl chloroformate (4) (2.46 g, 10 mmol) were refluxed for 5 h under an argon atmosphere. The reaction was cooled, solvent evaporated in vacuo and the residue crystallized from benzene/hexane (1:4). Yield, 2.32 g (96%); m.p. 99-101 °C; NMR ($CDCl_3$) δ 2.88 (d, 3H, NHC<u>H</u>3), 5.1 (br. s, 1H, N<u>H</u>), 6.79 (s, 1H, C<u>H</u>Cl); EI/MS, mass for $C_4H_5NO_2Cl_4$: calculated, 238.9074; found 238.9089; TLC (silica) hexane/CH_2Cl_2 (65:35), rf 0.52.

1,2,2,2-Tetrachloroethyl N-methyl-N-nitrosocarbamate (6)

Under an argon atmosphere, a stirred solution of N_2O_4 (240 mg, 2.6 mmol) in CCl_4 (10 ml) at -60 °C was treated with NaOAc (640 mg, 7.8 mmol). The mixture was warmed to 0 °C and a solution of 5 (482 mg, 2 mmol) in CCl_4 (10 ml) was added. After stirring for an additional 20 min, the reaction mixture was poured onto ice and the resulting organic layer separated, washed with water (3 x 5 ml), dried (sodium sulfate) and then evaporated to yield the nitrosocarbamate 6 as yellow oil. Yield, 390 mg (76%); NMR ($CDCl_3$) δ 3.30 (s, 3H, C<u>H</u>$_3$) and 7.18 (s, 1H, C<u>H</u>Cl); EI/MS, mass for $C_4H_4N_2O_3Cl_4$: calculated, 267.9024; found 267.9000; TLC (silica) hexane/CH_2Cl_2 (3:2), rf 0.6.

Preparation of Labeled Materials

1,2,2,2-Tetrachloroethyl N-[^{3}H]methylcarbamate (5)

[^{3}H]Methylamine hydrochloride (48 Ci/mmol) and methylamine hydrochloride (70.2 mg, 1.04

mmol) in dry dioxane (2 ml), and 1,2,2,2-tetrachloroethyl chloroformate (4) (400 μl, 1.04 mmol) were refluxed for 5 h under an argon atmosphere. The reaction was cooled, solvent evaporated in vacuo and the residue crystallized from benzene/hexane (1:4). Yield, 96% (specific activity, 30.1 μCi/mmol); NMR ($CDCl_3$) δ 2.9 (d, 3H, $NHCH_3$), 5.1 (br. s, 1H, NH), 6.8 (s, 1H, CHCl); TLC (silica) hexane/CH_2Cl_2 (65:35), rf 0.52.

1,2,2,2-Tetrachloroethyl N-[^{3}H]methyl-N-nitrosocarbamate (6)

Under an argon atmosphere, a stirred solution of N_2O_4 (133 mg, 1.45 mmol) in CCl_4 (5 ml) at -60 °C was treated with NaOAc (357 mg, 4.35 mmol). The mixture was warmed to 0 °C and a solution of [^{3}H]5 (232 mg, 0.96 mmol) in CCl_4 (5 ml) was added. After stirring for an additional 20 min, the reaction mixture was poured onto ice, and the resulting organic layer separated, washed with water (3 x 5 ml) and dried (Na_2SO_4). After filtration and concentration, nitrosocarbamate [^{3}H]6 was obtained as a yellow oil. Yield, 76% (specific activity, 28.5 μCi/mmol); TLC (silica) hexane/CH_2Cl_2 (3:2), rf 0.6.

2,7-Diamino-9-[p-[[2-[N-nitroso-N-methylcarbamoyl)amino]ethyl]carbamoyl]phenyl]-10-methylphenathridinium chloride (7)

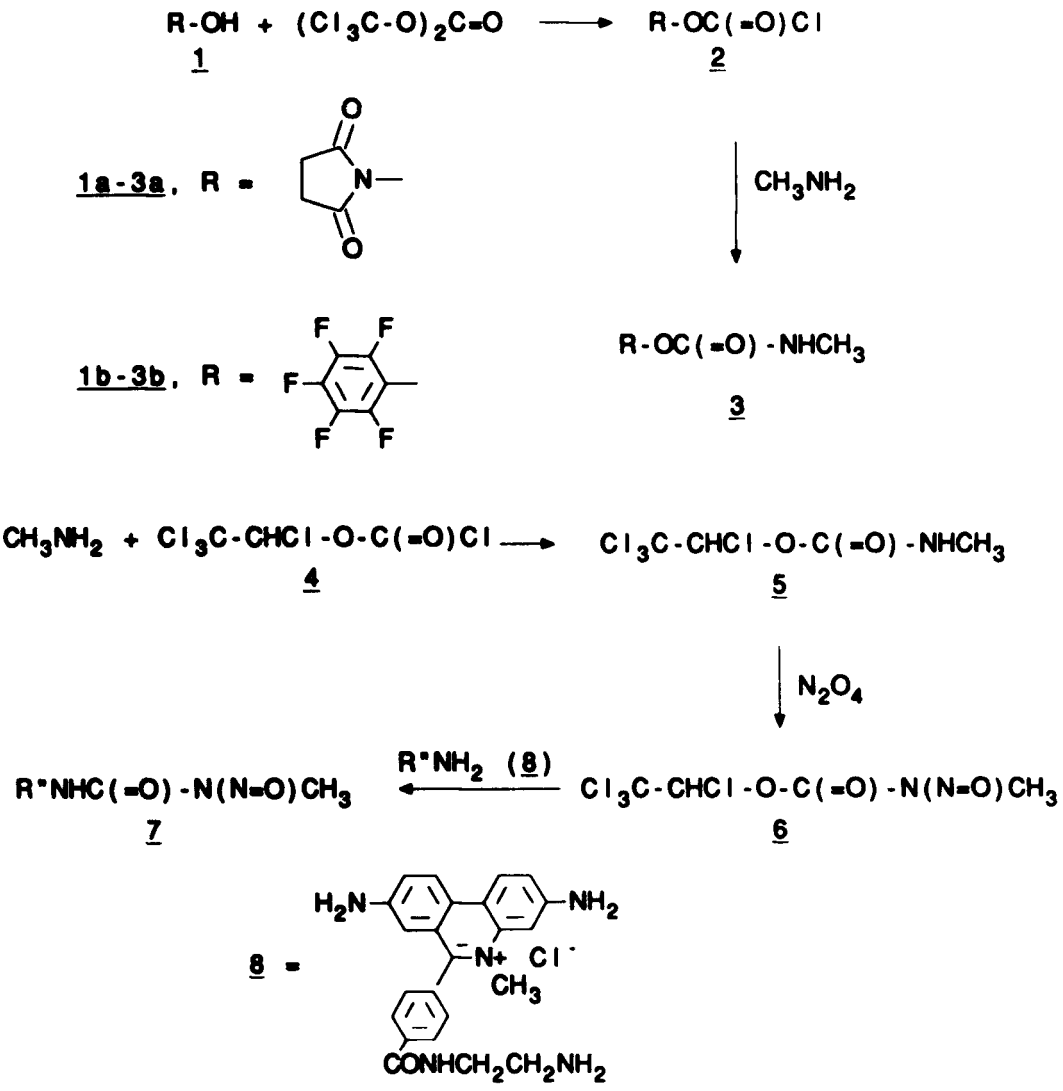
To a cold solution of [^{3}H]6 (23 mg, 88.2 μmol, 28.47 μCi/mmol) in anhydrous DMF (5 ml) under an Ar atmosphere was slowly added via a syringe a mixture of the methidium amine hydrochloride 8 (4) (27 mg, 58.8 μmol) and diisopropylethylamine (50 μl, 0.264 mmol) in dry DMF (5 ml). The solution was stirred at -5 °C for 7 h, allowed to warm to room temperature and then concentrated in vacuo at 35 °C. The residue was dissolved in anhydrous MeOH (1.5 ml) and then EtOAC (15 ml) added and the resulting solid collected by filtration. This solid was thoroughly washed with EtOAc and then dried in vacuo to afford 25 mg of purple solid. Yield, 60% (specific activity, 28.2 μCi/mmol); TLC (silica) AcOH/nBuOH/H_2O (1:4:2.5), rf 0.52. This labeled material co-migrates on TLC with cold authentic nitrosourea 7 (4).

RESULTS AND DISCUSSION

In previous reports describing the synthesis of activated N-methylcarbamates (3), methylisocyanate, prepared by the reaction of methylamine and triphenylphosphinedibromide was

used. Because the yield by this route is poor and because of the number of steps involved, this method is not particularly attractive for the preparation of radiolabeled material. To overcome the use of methylisocyanate, the problem of regioselective N-nitrosation of ureas, and the uneconomical yields, other synthetic routes were investigated, specifically the use of different chloroformate analogues (**Scheme 1**).

Scheme 1



LEGEND FOR SCHEME 1 - Synthesis of activated carbamates and N-nitroso derivatives.

Chloroformate derivatives (2) of N-hydroxysuccinimide (1a) and pentafluorophenol (1b) were synthesized by their reaction with bis(trichloromethyl)carbonate in the presence of diisopropylethylamine at 0 °C (Scheme 1). The resulting N-succimidyl chloroformate (2a) and pentafluorophenyl chloroformate (2b), were of sufficient purity to be used without isolation. The reactions of 2a and 2b with methylamine in the presence of diisopropylamine gave N-methyl succimidylcarbamate (3a) and N-methyl pentafluorophenylcarbamate (3b) in 65 and 35% yields, respectively. However, using 1,2,2,2-tetrachloroethyl chloroformate (4) (6) and methylamine in refluxing dioxane, the yield of the N-methylcarbamate (5) was ~95%. Besides the excellent yield of carbamate 5, this method has the advantage of avoiding the synthesis of the unstable chloroformate intermediate. The synthesis was repeated with [^{3}H]methylamine to afford 1,2,2,2-tetrachloroethyl N-[^{3}H]methylcarbamate (11) in 96% yield, specific activity, 30.1 μCi/mmol) (Scheme 1). This carbamate was then converted to the nitrosocarbamate analogue 6 by treatment with N_2O_4 (7) in 76% yield. In turn, the 1,2,2,2,-tetrachloroethyl N-methyl-N-nitrosocarbamate (6) was reacted with 8, an amino functionalized derivative of methidium chloride (4), to prepare the desired [^{3}H-methyl]-N-methylnitrosourea derivative (7).

CONCLUSION

The synthesis of the activated carbamate 1,2,2,2-tetrachloroethyl N-[^{3}H]-methyl-N-carbamate using [^{3}H]methylamine and 1,2,2,2-tetrachloroethyl chloroformate is reported. The use of this chloroformate intermediate (prepared in ~95% yield) and the resulting activated carbamate (prepared in 76% yield) has clear advantages over alternative intermediates in terms of yield and ease of preparation.

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REFERENCES

1. For review see - Nitrosoureas: Current Status and New Developments, Prestayko A. W., Crooke S. T., Baker L. H., Carter S. K. and Schein P. S. (Eds.) Academic Press, New York, 1981
2. Preussmann R. and Stewart B. W.- Chemical Carcinogens, Searle C. E. (Ed.), American Chemical Society Monograph 182, pp 643-828, 1984
3. Martinez J., Oiry J., Imbach J. L. and Winterniz F.- J. Med. Chem. 25: 178 (1982)
4. Konakahara T., Wurdeman R. L. and Gold B.- Biochemistry 27: 8606 (1988)
5. Church K. M., Wurdeman R. L., Zhang Y., Chen F.-X. and Gold B. - Biochemistry 29: 6827 (1990)
6. Barcelo G., Senet J.-P. and Sennyey G.- Synthesis 11: 1027 (1987)
7. White E. H. - J. Am. Chem. Soc. 77: 6008 (1955)